THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 270, No. 37, Issue of September 15, pp. 21902–21906, 1995
Printed in U.S.A.

Aggregation of IgE Receptors in Rat Basophilic Leukemia 2H3 Cells Induces Tyrosine Phosphorylation of the Cytosolic Protein-tyrosine Phosphatase HePTP*

(Received for publication, April 28, 1995, and in revised form, June 28, 1995)

Mark Swieter‡, Elsa H. Berenstein, William D. Swaim, and Reuben P. Siraganian

From the Laboratory of Immunology, NIDR, National Institutes of Health, Bethesda, Maryland 20892

The cDNA encoding the rat equivalent of the human hematopoietic tyrosine phosphatase, also known as leukocyte phosphatase, was isolated from a rat basophilic leukemia mast cell cDNA library. By two-dimensional electrophoresis, the protein expressed in the mast cells was of a size (40 kDa) and pI (6.9) predicted from the deduced amino acid sequence. Thus, although previously shown to be preferentially expressed in T cells and B cells, the phosphatase is also found in mast cells. By immunofluorescence microscopy, rat hematopoietic tyrosine phosphatase localized to discrete, globular compartments within the cytoplasm and was not found either in the nucleus or associated with the cell surface membrane. Aggregation of high affinity IgE receptors in the mast cells induced tyrosine phosphorylation of the phosphatase. The tyrosine phosphorylation was mimicked by stimulation with calcium ionophore A23187 but not by direct activation of protein kinase C. Since phosphorylation of the phosphatase was dramatically reduced when the cells were activated in Ca^{2+}-free media, it is dependent on a rise in intracellular Ca^{2+}. These data strongly suggest that hematopoietic tyrosine phosphatase may be involved in the IgE receptor-mediated signaling cascade.

Mast cells and basophils play a central role in allergic and inflammatory reactions. They express high affinity IgE receptors (FcεRI)[1] on their cell surfaces that, when aggregated, initiate biochemical events that lead to the release of inflammatory mediators. In the rat basophilic leukemia (RBL-2H3) mast cell line, aggregation of FcεRI induces activation of phospholipases A_2, C, and D, an increase in intracellular Ca^{2+} concentration, and activation and translocation of protein kinase C from the cytosol to the plasma membrane (1–5). In addition, numerous proteins become tyrosine phosphorylated following receptor aggregation. These include the β and γ subunits of the receptor, phospholipase C-γ1, Vav, Nck, and paxillin; protein-tyrosine kinases such as Lyn, Syk, Fak, and Btk; and other unidentified proteins (6–21).

Regulation of the level of tyrosine phosphorylation of proteins through dephosphorylation is an important aspect of the signal transduction process. For example, the protein-tyrosine phosphatase (PTP) CD45 is essential for signaling from receptors on T cells, B cells, and mast cells (22–26). However, we found that CD45 is absent from several RBL-2H3 cell variants that have normal signaling through their FcεRI.[2] Therefore, we used a molecular cloning approach to identify other PTPs that may be involved. The rat equivalent of human hematopoietic tyrosine phosphatase (HePTP),[3] also known as leukocyte protein-tyrosine phosphatase (LC-PTP; Refs. 27 and 28) was isolated from an RBL-2H3 cell cDNA library. HePTP mRNA was present only in RBL-2H3 mast cells, the YAC-1 T cell line, and the thymus in Northern blots. Moreover, the protein became tyrosine phosphorylated upon aggregation of the FcεRI in RBL-2H3 cells. This phosphorylation was Ca^{2+}-dependent and accordingly would be considered a "late" event in the activation process. Thus, HePTP may be involved in the signaling cascade initiated by IgE receptor aggregation.

EXPERIMENTAL PROCEDURES

Molecular Cloning—Two degenerate primers were designed based on common sequences in the catalytic domains of several PTP ((His/Asp)-Phe-Trp-(Arg/Lys/Leu/Glu)-Met-(Val/Cys/Ile)-Trp-(Glu/Gly) to Val-His-Cys-Ser-Ala-Gly-(Val/Ile)-Gly) (29). Forward 5′-GC GAA TTC (C/G)A(T/C) TT(C/T) TGG I(G/A/T)I ATG (G/A)TI TGG (G/C)A-3′ and reverse 5′-GC GAA TTC CCI A(T/C)I CCI GC(G/A) CT(G/A) TGI AC-3′ oligonucleotide primers were synthesized on an Applied Biosystems Inc. 392 DNA/RNA synthesizer. RBL-2H3 cell cDNA, prepared as described previously (30), served as template in PCR reactions. The PCR products were subcloned into the *Eco*RI site of pBluescript SK (Stratagene, La Jolla, CA), and the sequences of both strands were determined by automated sequence analysis (Applied Biosystems Inc., Foster City, CA). The HePTP PCR product was used to screen a previously characterized RBL-2H3 cell cDNA λgt11 library (30). Positive plaques were purified, their inserts were subcloned in pBluescript SK, and the sequence of both strands was determined by automated sequencing.

The 5′-end was generated using the 5′ rapid amplification of cDNA ends system of Life Technologies, Inc. Poly(A)$^+$ RNA from RBL-2H3 cells and the PTP gene-specific primer 5′-TAGAGTCCAGCGTGTA-3′ corresponding to nucleotides 367–352 in the rat PTP were used. The rapid amplification of cDNA ends products were subcloned into pBluescript SK and sequenced on both strands by automated sequence analysis.

Antibody Preparation—A rat PTP-specific peptide comprising amino acids 121–134 (SKDRYKTILPNPQS) with a cysteine residue at the carboxyl end was synthesized by Chiron Mimotopes (Emeryville, CA). Antibodies were generated in rabbits as described previously (15). Some affinity-purified anti-PTP antibodies were biotinylated using NHS-LC-biotin (Pierce) according to the manufacturer's recommendations.

Two-dimensional Gel Electrophoresis—Whole cell lysates from both

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) U28356.

‡ To whom correspondence should be addressed: Laboratory of Immunology, Bldg. 10, Rm. 1N106, NIDR, NIH, Bethesda, MD 20892. Tel.: 301-496-5105; Fax: 301-480-8328.

[1] The abbreviations used are: FcεRI, high affinity IgE receptor; PCR, polymerase chain reaction; PTP, protein-tyrosine phosphatase(s); LC-PTP, leukocyte protein-tyrosine phosphatase; HePTP, hematopoietic tyrosine phosphatase; PAGE, polyacrylamide gel electrophoresis; RBL-2H3, rat basophilic leukemia 2H3 cells.

[2] M. Swieter, E. H. Berenstein, and R. P. Siraganian, submitted for publication.

[3] Because the cDNA sequence for human HePTP was reported before that of human LC-PTP, HePTP was chosen to designate the rat protein in this manuscript.

nonstimulated and activated RBL-2H3 cells were used as described previously (20). Blots were probed with 100 ng/ml biotinylated anti-rat HePTP and 10 pg/ml horseradish peroxidase-conjugated streptavidin (Pierce).

Immunofluorescence Microscopy—Immunofluorescence microscopy was as described previously (20) using 10 μg/ml nonimmune rabbit IgG or rabbit anti-PTP (described above) and fluorescein isothiocyanate-conjugated $F(ab')_2$ donkey anti-rabbit IgG (Jackson ImmunoResearch).

Cells and Cell Activation—RBL-2H3 cells were maintained as monolayer cultures as described previously (31). For activation, 150-mm diameter Petri dishes were seeded with 1.2–2 $\times$ 10^7 cells. After overnight culture, the cell monolayers were washed twice with 30 ml of phosphate-buffered saline at room temperature. The cells were then incubated at 37 °C for 10 min unless otherwise indicated in 10 ml of Eagle's minimum essential medium with Earle's salts containing 0.1% bovine serum albumin, 10 mM Tris (pH 7.4), and 0.03 μg/ml anti-FcϵRI monoclonal antibody BC4; 0.5 μM calcium ionophore A23187; or 40 nM phorbol 12-myristate 13-acetate. The supernatants were removed and assayed for histamine by automated analysis (32).

Immunoprecipitations—After activation, the monolayers were washed once with 10 ml of ice-cold phosphate-buffered saline containing protease inhibitors (concentrations as in lysis buffer), solubilized in 1 ml of lysis buffer (3% Brij 96, 20 mM Tris, pH 7.4, 100 mM NaCl, 1 mM Na_3VO_4, 2 mM phenylmethylsulfonyl fluoride, 90 milliunits/ml aprotinin). Postnuclear lysates were precleared 1 h at 4 °C with protein A-agarose, and proteins were immunoprecipitated at 4 °C for 1 h with 5 μg of affinity purified anti-rat HePTP antibodies bound to protein A-agarose. The beads were washed 7 times with ice-cold lysis buffer, and proteins were eluted by boiling 5 min in SDS-PAGE sample buffer. Brij 96, protease inhibitors, and protein A-agarose beads were from Sigma.

Immunoblotting—Whole cell lysates and immunoprecipitated proteins were separated by SDS-PAGE using 4–20% linear gradient gels (Novex) under reducing conditions. Separated proteins were electrotransferred to polyvinylidene difluoride membranes (Immobilon) and blocked by overnight incubation in 4% protease-free bovine serum albumin (Intergen). For detection of tyrosine-phosphorylated proteins, membranes were probed with 40 ng/ml anti-phosphotyrosine mAb PY-20 coupled to horseradish peroxidase (ICN). Signals were detected by chemiluminescence using a Renaissance kit (DuPont NEN) and Kodak X-Omat radiographic film (Eastman Kodak Co.). Membranes were then stripped of antibodies according to the protocol of the Amersham ECL kit and reprobed for PTP with 100 ng/ml biotinylated anti-rat HePTP IgG followed by 10 pg/ml horseradish peroxidase-conjugated streptavidin (Pierce).

RESULTS

Isolation and Analysis of Rat HePTP cDNA—Part of the catalytic domain of rat HePTP was amplified from RBL-2H3 cell cDNA using reverse transcriptase-based PCR and degenerate oligonucleotide primers. The amplified product was used to screen an RBL-2H3 cDNA λgt11 library. Out of 5 $\times$ 10^5 plaques surveyed, 5 hybridized to the probe. The 5 clones yielded similar sized cDNA, one of which was completely sequenced on both strands. Because the 5′-end (nucleotides 1–226) of the rat HePTP cDNA was missing, the remaining four clones, the RBL-2H3 cell λgt11 library, and a rat spleen library were all screened by PCR using phage-specific and HePTP-specific primers. However, no new products were found. Therefore, the 5′-end was generated using the 5′ rapid amplification of cDNA ends technique.

The whole cDNA sequence obtained contains a single open reading frame encoding a putative protein 359 amino acids in length (Fig. 1). The presumptive initiation codon (nucleotides 107–109) is surrounded by a consensus Kozak sequence and is preceded by stop codons in all three reading frames. The rat and human sequences (27, 28) share 77 and 91% identity at the nucleotide and amino acid levels, respectively. By Northern blotting, rat HePTP was found to be restricted in its distribution to T cells and RBL-2H3 cells (Fig. 2).

Rat HePTP Protein Expression and Distribution in RBL-2H3 Mast Cells—To determine if RBL-2H3 cells expressed the HePTP protein, cell lysates were separated by one- and two-dimensional electrophoresis and analyzed by immunoblotting. The rat HePTP had a molecular mass of ~40 kDa and an isoelectric point of ~6.9 (Fig. 3), in agreement with values predicted from the deduced amino acid sequence. The deduced amino acid sequence also suggested that the phosphatase was a cytosolic protein. Therefore, we examined its subcellular localization by immunofluorescence microscopy (Fig. 4). In unstimulated RBL-2H3 cells, the protein was evenly distributed throughout the cytoplasm, with little if any appearing at the plasma membrane or in the nucleus (Fig. 4*B*). However, after stimulation through the high affinity IgE receptors, the cells spread (Fig. 4*D*) and it became apparent that the phosphatase was localized to globular-shaped subcellular compartments (Fig. 4*E*). As the immunofluorescent globules were more abundant than the secretory granules, it is unlikely that HePTP is present in secretory granules.

```
                                                                AGACGGACTGACAGACAGCTGGCAAGAGGCA    31
GCCCTGGGGACATAGCTGCTTCAGCAGGCCTCATGGCTGAGTGAGCCTCCCTTGGGCCCAGCAACCTACCCCAGC   106

M  V  Q  A  C  E  G  R  S  R  A  Q  L  P  T  L  S  L  G  A  D  M  T  Q  P
ATGGTCCAGCCCTGTGAGGGGCGCTCCAGAGCACAGCTGCCGACCTTGTCTTGGGGGCAGACATGACTCAGCCT   181

P  P  A  K  A  P  A  K  K  H  V  R  L  Q  E  R  R  G  S  S  V  A  L  M  L
CCACCCGCCAAAGCTCCAGCCAAGAAGCATGTGCGGCTGCAGGAGAGAAGGGGCTCCAGTGTGGCTCTGATGCTG   256

D  V  R  S  L  G  T  V  E  P  I  C  S  V  N  T  P  R  E  V  T  L  H  F  L
GATGTGCGGTCACTGGGCACTGTCGAGCCCATCTGCTCAGTGAACACACCCCGTGAAGTCACCCTGCACTTCCTG   331

R  T  A  G  H  P  L  T  R  W  T  L  Q  H  Q  P  P  S  P  K  Q  L  E  E  E
CGCACAGCCGGGCACCCCCTTACACGCTGGACTCTACAGCACCAGCCTCCCAGCCCCAAGCAGCTAGAAGAGGAA   406

F  L  K  I  P  S  N  F  V  N  P  E  D  L  D  I  P  G  H  A  S  K  D  R  Y
TTCTTGAAGATCCCCTCAAACTTTGTCAACCCTGAAGACCTGGATATCCCTGGCCATGCCTCCAAAGACCGATAC   481

K  T  I  L  P  N  P  Q  S  R  V  C  L  G  R  A  H  S  Q  E  D  S  D  Y  I
AAGACCATCTTGCCAAATCCCCAGAGCCGTGTCTGTCTTGGCCGGCCACACAGCCAGGAGGACAGCGACTACATC   556

N  A  N  Y  I  R  G  Y  D  G  K  E  K  V  Y  I  A  T  Q  G  P  M  P  N  T
AATGCCAATTACATCCGGGGCTATGATGGAAAGGAGAAGGTCTACATTGCTACCCAGGGCCCCATGCCCAACACT   631

V  A  D  F  W  E  M  V  W  Q  E  D  V  S  L  I  V  M  L  T  Q  L  R  E  G
GTAGCAGACTTCTGGGAGATGGTGTGGCAGGAAGATGTATCCCTCATTGTCATGCTCACCCAGCTCCGAGAGGGC   706

K  E  K  C  V  H  Y  W  P  T  E  E  E  A  Y  G  P  F  Q  I  R  I  Q  G  M
AAGGAGAAATGTGTACACTACTGGCCCACAGAAGAGGAAGCCTATGGGCCCTTCCAGATCCGCATCCAGGGCATG   781

K  E  H  P  E  Y  T  V  R  H  L  T  I  Q  H  Q  Q  E  C  R  S  V  K  H  I
AAACAGCACCCAGAATATACTGTGAGGCACCTCACCATTCAGCACCAACAGGAATGCCGGTCAGTGAAACACATC   856

L  F  S  A  W  P  D  H  Q  T  P  E  S  A  G  P  L  L  R  L  V  A  E  V  E
CTCTTCTCAGCCTGGCCAGACCACCAGACGCCAGAATCAGCCGGGCCCCTGCTGCGCCTGGTGGCTGAGGTGGAG   931

T  P  E  T  A  A  N  S  G  P  I  V  V  H  C  S  A  G  I  G  R  T  G  C  F
ACCCCAGAGACAGCTGCCAACTCCGGGCCTATAGTGGTCCACTGCAGCGCAGGGATCGGTCGGACAGGCTGCTTC  1006

I  A  T  R  I  G  C  Q  Q  L  K  A  R  G  E  V  D  I  L  G  I  V  C  Q  L
ATCGCCACCCGAATCGGCTGCCAGCAGCTGAAGGCCCGAGGGGAGGTGGACATTCTGGGCATTGTGTGCCAACTT  1081

R  L  D  R  G  G  M  I  Q  T  A  E  Q  Y  Q  F  L  H  H  T  L  A  L  Y  A
CGCCTAGACAGAGGAGGCATGATCCAGACGGCGGAGCAGTACCAGTTCCTACACCACACTTTGGCCCTGTATGCA  1156

A  Q  L  P  P  E  T  D  P  *
GCCCAGCTGCCCCCGGAGACCGACCCCTGACCCCTGACCCCTGACCCCTGACCCTCCACCAGCCCAGG  1231
GACCAACCTTCTCTCAGGTCAGGGACAGTGGGTCTGGGGAAGTGGCCTGTGCGCTCTGGCATGGCTTCTGCATAA  1306
GCACAGGAAAGGGAGCCTTGGAAGCAAGAATGTTTACAGTCTCAGGAAACTGCCCCAGCAAGCAACAGGGGCCA  1381
GGCCGTAGGTCTTCGCCACAGACCATGACTCTGCCTTCTTCAGTGGCCCCCGGTGGACTCCAGGGGAGCTTCCCT  1456
AAGTGGCTCTGAACTTACAGATAGGAGCTGGTCCTTGAAATGGATTGAGAAAGCGGCTGAGGCACTGGCTTGTCT  1531
TTGTGCTTCCTGTGGGCTCCTTTCCTTGTCGGTGACACAGAGTCAGTCCTTTGCTGCCATTGTAGAAGCAGACAG  1606
AATGTTCCTGAAAGCCATTCCAGGTAGCCCTGCCGTTGGGGCTGCAGAGATGCTGATCGTAAGATCGTCTTCCGG  1681
TAAGGCCTTGCCCACACACAGGCCAGATGCAAGCGCAGGGAAAATCCCAGTCTGACCTTGGTCAAATCCTCTCTA  1756
TTCTACATAGACTGCTCCTCCCCATACACCGGGGGGTGCTGGATGACCCGCTCAGAAGAGAATAAAGCCTTGGT  1831
TGGCCCCCCTCCACACCTGTGGTAAAAGGAGACGCAGAGGTAACCAGGTCCAGCAGTTAGGAATCCCTGGAAGAG  1906
CCCAGGTGTGATGACCTACGTACTCATCCTCCAGCACTCAGGAGTAGAAGCAGGAGGATTGCTGCAAATTTGAGG  1981
CCAGCTAGATCTATATTTTGAGTACCAGGCCAGACAGGACTACATAGCAAG                            2032
```

FIG. 1. **Nucleotide and predicted amino acid sequence of rat HePTP cDNA.** The nucleotide sequence is numbered on the *right*. The predicted amino acid sequence of the coding region is in single letter codes *above* the nucleotide sequence.

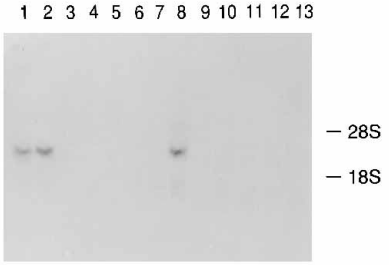

FIG. 2. **Northern blot analysis of HePTP mRNA expression in cultured cell lines and rat tissues.** Northern blotting of 30 μg/lane total RNA was with a 180-base pair fragment (nucleotides 227–406) from the 5′-noncatalytic domain of the coding sequence. Samples were derived from: YAC-1 mouse T cell line (*1*), RBL-2H3 cells (*2*), hindbrain (*3*), olfactory bulb (*4*), cerebellum (*5*), frontal lobe (*6*), liver (*7*), thymus (*8*), spleen (*9*), kidney (*10*), heart (*11*), testis (*12*), lung (*13*).

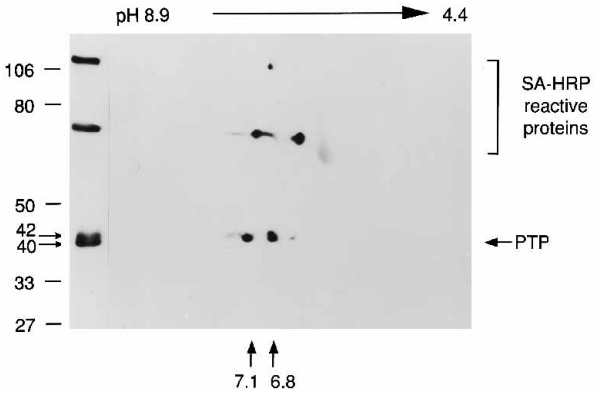

FIG. 3. **One- and two-dimensional analysis of HePTP in RBL-2H3 mast cells.** Cell lysates were resolved either by one- (*left single lane*) or by two-dimensional analysis, electrotransferred to nitrocellulose membranes and blotted with biotinylated anti-HePTP antibodies and horseradish peroxidase-conjugated streptavidin (*SA-HRP*). The proteins at the *top* of the blot were recognized by horseradish peroxidase-conjugated streptavidin in the absence of anti-HePTP antibody (data not shown). In cells solubilized for two-dimensional analysis in urea lysis buffer, the HePTP was identified as a doublet, whereas by one-dimensional SDS-PAGE, it migrated as a single band.

FcεRI Aggregation Induces Tyrosine Phosphorylation of HePTP—A prominent feature of signaling in RBL-2H3 cells is the phosphorylation of numerous proteins on tyrosine residues. To determine if rat HePTP also became tyrosine-phosphorylated in IgE receptor-activated cells, HePTP was immunoprecipitated from resting and from stimulated cells, separated by SDS-PAGE, and immunoblotted with anti-phosphotyrosine antibodies. As shown in Fig. 5, HePTP was at best only weakly tyrosine phosphorylated in nonactivated cells. By contrast, after FcεRI aggregation, HePTP underwent time-dependent tyrosine phosphorylation (Fig. 5). Phosphorylation was detectable by 1 min, peaked at 5–10 min, and declined thereafter. It did, however, remain detectable up to 60 min, the longest time point tested.

Because rat HePTP became tyrosine-phosphorylated upon FcεRI aggregation, it was possible that the phosphatase might interact with the receptor or one of the receptor-associated proteins. However, in immunoblotting experiments, we could not detect any HePTP in immunoprecipitates of Lyn, Syk, or of the IgE receptors, and conversely, we found no evidence of Lyn, Syk or of the IgE receptor subunits in HePTP immunoprecipitates. Thus, although HePTP is tyrosine-phosphorylated by receptor aggregation, it does not appear to physically associate with either Lyn, Syk, or the IgE receptors.

Characteristics of the Tyrosine Phosphorylation of HePTP—Some proteins are phosphorylated on tyrosine very early after IgE receptor aggregation, and others are phosphorylated only after a rise in intracellular Ca^{2+} and/or after activation of protein kinase C (9, 33, 34). Experiments therefore examined the relationship between tyrosine phosphorylation of the HePTP, Ca^{2+} influx, and activation of protein kinase C. Stimulation with the Ca^{2+} ionophore A23187 was as effective as FcεRI cross-linking at inducing tyrosine phosphorylation of HePTP (Fig. 6). In contrast, direct activation of protein kinase C by the addition of phorbol 12-myristate 13-acetate failed to do so (Fig. 6). Therefore, an increase in intracellular calcium but not PKC activation can directly stimulate tyrosine phosphorylation of HePTP.

To more fully explore the role of extracellular calcium in the FcεRI- and ionophore-induced tyrosine phosphorylation of HePTP, RBL-2H3 cells were first rinsed and then activated in Ca^{2+}-free media containing EDTA (Fig. 7, *lanes 4–6*). In the absence of extracellular Ca^{2+}, tyrosine phosphorylation of HePTP was substantially diminished when compared with controls (*lanes 6* and *3*). However, because some tyrosine-phosphorylated HePTP was still detected after receptor aggregation or after ionophore stimulation in Ca^{2+}-free media (*lanes 5* and *6*), the small rise in intracellular Ca^{2+} due to release from intracellular compartments probably contributed the requisite divalent ions. Identical results were obtained when the concentration of EDTA in the wash and incubation media was 40 μM or 4 mM. Thus, FcεRI-mediated tyrosine phosphorylation of HePTP is a Ca^{2+}-dependent process and is one of the late signaling events.

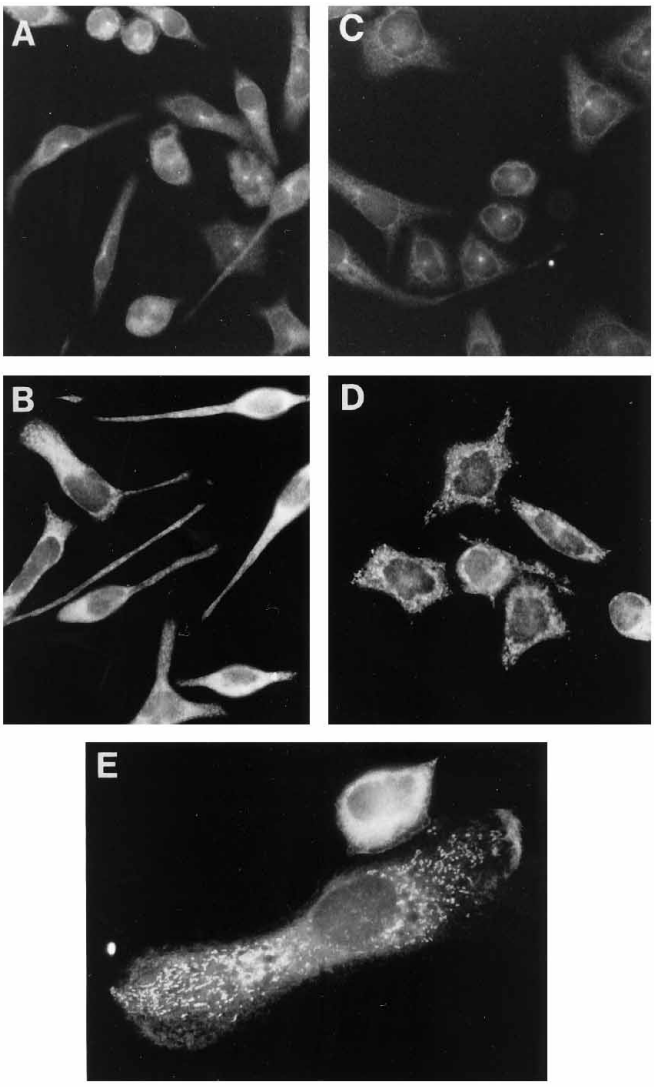

FIG. 4. **Immunofluorescence localization of HePTP in resting and activated RBL-2H3 mast cells.** RBL-2H3 cells were grown on glass coverslips in 6-well tissue culture dishes. Following methanol permeabilization, the cells were incubated with 10 μg/ml normal rabbit IgG (*A* and *C*) or affinity-purified rabbit anti-rat HePTP IgG (*B*, *D*, and *E*). Unstimulated RBL-2H3 cells (*A* and *B*) are characteristically rounded with a bipolar shape. After activation (*C–E*), they spread out along the substratum and take on a more fibroblast-like appearance. HePTP is distributed throughout the cytoplasm, but it is not found either in the nucleus or at the cell surface (*B*, *D*, and *E*). After cell activation, HePTP localizes to globular-shaped subcellular compartments. Original magnification of *panels A–D* is 40×, of *panel E* is 60×.

DISCUSSION

The cDNA we isolated is the rat equivalent of the human PTP HePTP or LC-PTP (27, 28). While the two human sequences are nearly identical, they do differ in one major respect: the location of the presumptive translation initiation

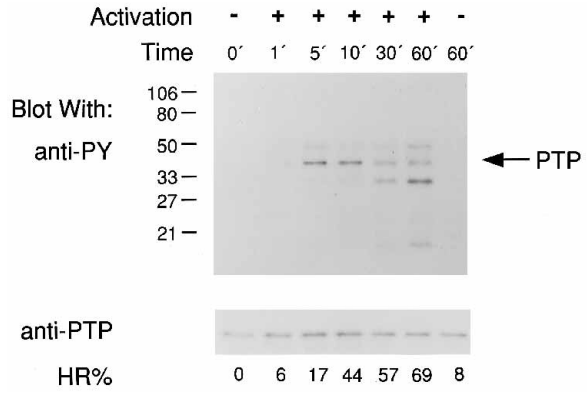

FIG. 5. **Time course of FcεRI-induced HePTP tyrosine phosphorylation.** Monolayer cultures of RBL-2H3 cells were stimulated with 30 ng/ml anti-receptor monoclonal antibody BC4 at 37 °C for the indicated times. Lysates were immunoprecipitated with anti-rat HePTP antibodies, and the precipitated proteins analyzed by immunoblotting with anti-phosphotyrosine antibodies (*upper panel*). The blots were stripped and assayed for HePTP to confirm equivalent loading (*lower panel*). Each lane represents the precipitate from 7.5×10^6 cells. Percent histamine release (*HR%*) results are at the *bottom* of each *lane*. *Arrow* indicates position of HePTP.

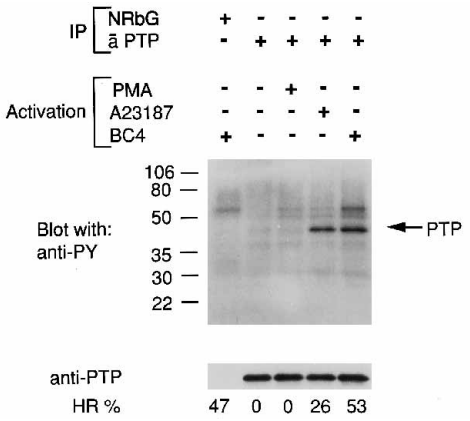

FIG. 6. **HePTP is tyrosine-phosphorylated by calcium ionophore A23187 but not by phorbol 12-myristate 13-acetate.** RBL-2H3 cells were stimulated for 10 min with anti-receptor monoclonal antibody BC4 (*BC4*), phorbol 12-myristate 13-acetate (*PMA*), calcium ionophore A23187, or buffer alone. Lysates were immunoprecipitated with normal rabbit IgG or anti-rat HePTP antibodies and analyzed by immunoblotting with anti-phosphotyrosine (*upper panel*). The blots were stripped and assayed for HePTP to confirm equivalent loading (*lower panel*). Each *lane* represents the precipitate from 7.5×10^6 cells. Percent histamine release (*HR%*) results are at the *bottom* of each *lane*. *Arrow* indicates position of HePTP.

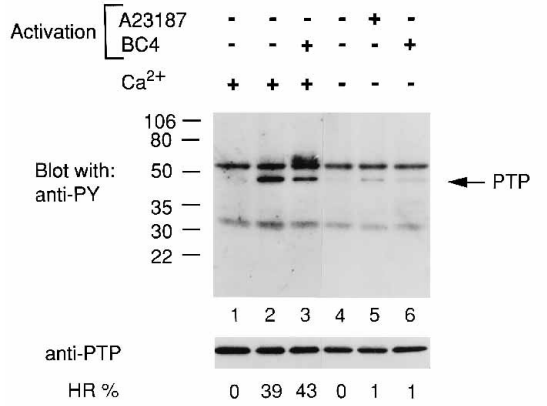

FIG. 7. **Importance of protein kinase C and Ca^{2+} in the tyrosine phosphorylation of HePTP.** RBL-2H3 cells were incubated for 10 min with buffer alone (*lanes 1* and *4*), with calcium ionophore A23187 (*lanes 2* and *5*), or with anti-FcεRI monoclonal antibody BC4 (*lanes 3* and *6*). In some cases (*lanes 4–6*), monolayer cultures were washed with Ca^{2+}-free medium containing 40 μM EDTA and then activated in the same medium. Lysates were immunoprecipitated with anti-rat HePTP antibodies and analyzed by immunoblotting with anti-phosphotyrosine (*upper panel*). The blots were stripped and assayed for HePTP to confirm equivalent loading (*lower panel*). Each lane represents the precipitate from 7.5×10^6 cells. Percent histamine release (*HR%*) results are at the *bottom* of each *lane*. *Arrow* indicates position of HePTP.

codon. The cDNA sequence of LC-PTP, which was confirmed by genomic cloning, suggests an open reading frame that begins at nucleotide 105 (LC-PTP numbering). In contrast, the cDNA sequence reported for human HePTP is lacking a cytosine at nucleotide 115 (LC-PTP numbering), a position within the putative coding sequence. The cDNA sequence that we present here suggests that the translation initiation codon of rat HePTP is the same as that of human LC-PTP. Thus, it may be that the cDNA library clone from which the human HePTP sequence was obtained contained a deletion.

By Northern blotting, human HePTP was detected in T cells and B cells (27, 28). In the present experiments, we also found the rat equivalent of this PTP to be selectively expressed and now extend the list of cell types to include RBL-2H3 mast cells. It is interesting to note that T cells, B cells, and mast cells comprise a limited set of cells that express on their surfaces multisubunit immune response receptors (35). Perhaps HePTP functions in a receptor-dependent manner in each of these cell types.

The cytochemical distribution of rat HePTP is intriguing; it localizes to globular or elongated cytoplasmic elements. This suggests that the enzyme is compartmentalized to an organelle or to some subcellular specializations. We speculate that the NH_2-terminal noncatalytic domain of HePTP may play a role in targeting the enzyme to its intracellular locales. PTP1B (36) and *DPTP61F* (37) are nontransmembrane phosphatases that contain carboxyl-terminal sequences involved in directing these proteins to the endoplasmic reticulum. PTPMEG1 (38), PTPH1 (39), and PTPD1 (40) are other cytosolic PTP that contain amino-terminal sequences with homology to proteins that associate with the cytoskeleton. This has lead some to conjecture that these or other such PTP may be involved in focal adhesions (41). Although the amino terminus of HePTP does not share homology with cytoskeleton-associated proteins, we nonetheless examined adherent RBL-2H3 cells for colocalization of the enzyme to sites of cellular attachment to the substratum. By laser confocal microscopy, HePTP did not accumulate along the basal (adherent) surface of RBL-2H3 cells.[4] Thus, it is unlikely that HePTP is associated with focal adhesion sites in these cells.

The signaling process initiated by FcεRI aggregation involves tyrosine phosphorylation of several proteins. Here we report that FcεRI aggregation induces tyrosine phosphorylation of the cytosolic protein-tyrosine phosphatase, HePTP. The results suggest that the FcεRI-induced tyrosine phosphorylation of HePTP is dependent on an elevation in the intracellular Ca^{2+} concentration. First, cells activated through the IgE receptors in media lacking Ca^{2+} showed a dramatic diminution in the level of HePTP tyrosine phosphorylation. The residual low level of HePTP phosphorylation seen under these conditions may be attributed to the relatively small amount of calcium stored within intracellular compartments and released upon cell stimulation (42). Second, triggering of the cells with Ca^{2+} ionophore in either calcium containing or calcium-free media mimicked the results obtained by aggregating the FcεRI.

[4] M. Swieter, E. H. Berenstein, W. D. Swaim, and R. P. Siraganian, unpublished results.

Thus, elevated intracellular Ca^{2+} concentrations are needed for the tyrosine phosphorylation of HePTP.

The rise in intracellular Ca^{2+} concentration that results from receptor engagement in many cell types is accompanied by activation of protein kinase C (42). Optimal tyrosine phosphorylation of some mast cell proteins, such as the focal adhesion kinase ($p125^{FAK}$) and the cytoskeletal protein paxillin, require both protein kinase C activation and influx of extracellular calcium (16, 18). However, in the present experiments, direct activation of protein kinase C with 40 nM phorbol 12-myristate 13-acetate failed to elicit HePTP phosphorylation. Thus, tyrosine phosphorylation of HePTP can occur independent of protein kinase C activation.

The requirement for calcium mobilization in the tyrosine phosphorylation of HePTP indicates that it occurs late in the signaling cascade (43). That is, it is preceded by other events including tyrosine phosphorylation and activation of phospholipase C-γ1, Lyn, and Syk. The time course experiments showing that HePTP became tyrosine phosphorylated between 1 and 5 min after FcεRI aggregation also bear this out.

The SH2 domain-containing protein-tyrosine phosphatase, Syp (also referred to as PTP1-D or SH-PTP2), was shown to be tyrosine phosphorylated in response to epidermal growth factor and platelet-derived growth factor receptor activation (44, 45). It was also constitutively tryrosine-phosphorylated in cells transformed with v-Src (44), suggesting that the Src family of kinases may be involved in phosphorylating the PTP. Lyn is a Src family tyrosine kinase found in abundance in RBL-2H3 cells; it coimmunoprecipitates with the FcεRI, and is believed to be critically important in the IgE receptor-mediated signal transduction process (10, 21, 46, 47). However, we found no evidence that Lyn and HePTP interacted. Likewise, we found no evidence for an association between HePTP and the other FcεRI-associated protein-tyrosine kinase, Syk.

Because protein-tyrosine phosphorylation is a prominent feature of signaling through the IgE receptor in mast cells and basophils, protein-tyrosine phosphatases must play an important regulatory role. We have identified the rat equivalent of HePTP in RBL-2H3 cells, shown that it localizes to a cytoplasmic compartment, that it becomes tyrosine phosphorylated as a result of IgE receptor aggregation, and that this phosphorylation is dependent on Ca^{2+}. These results strongly suggest that HePTP may be involved in the IgE receptor-mediated signaling cascade in these cells.

Acknowledgments—We thank Drs. Majed Hamawy and Nick Ryba for helpful discussions and for reviewing this manuscript. We also thank Greta Bader for histamine analysis and Nikki Hayes for DNA sequence analysis.

REFERENCES

1. Beaven, M. A., Moore, J. P., Smith, G. A., Hesketh, T. R., and Metcalfe, J. C. (1984) *J. Biol. Chem.* **259,** 7137–7142
2. Garcia-Gil, M., and Siraganian R. P. (1986) *J. Immunol.* **136,** 259–263
3. Lin, P., Wiggan, G. A., and Gilfillan, A. M. (1991) *J. Immunol.* **146,** 1609–1616
4. Beaven, M. A., Rogers, J., Moore, J. P., Hesketh, T. R., Smith, G. A., and Metcalfe, J. C. (1984) *J. Biol. Chem.* **259,** 7129–7136
5. White, K. N., and Metzger, H. (1988) *J. Immunol.* **141,** 942–947
6. Benhamou, M., Gutkind, J. S., Robbins, K. C., and Siraganian, R. P. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 5327–5330
7. Paolini, R., Jouvin, M. H., and Kinet, J. P. (1991) *Nature* **353,** 855–858
8. Park, D. J., Min, H. K., and Rhee, S. G. (1991) *J. Biol. Chem.* **266,** 24237–24240
9. Benhamou, M., Stephan, V., Robbins, K. C., and Siraganian, R. P. (1992) *J. Biol. Chem.* **267,** 7310–7314
10. Eiseman, E., and Bolen, J. B. (1992) *Nature* **355,** 78–80
11. Hutchcroft, J. E., Geahlen, R. L., Deanin, G. G., and Oliver, J. M. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 9107–9111
12. Kawakami, T., Imagaki, N., Takei, M., Fukamachi, H., Coggeshall, K. M., Ishizaka, K., and Ishizaka, T. (1992) *J. Immunol.* **148,** 3513–3519
13. Li, W., Deanin, G. G., Margolis, B., Schlessinger, J., and Oliver, J. M. (1992) *Mol. Cell. Biol.* **12,** 3176–3182
14. Margolis, B., Hu, P., Katzav, S., Li, W., Oliver, J. M., Ullrich, A., Weiss, A., and Schlessinger, J. (1992) *Nature* **356,** 71–74
15. Benhamou, M., Ryba, N. J. P., Kihara, H., Nishikata, H., and Siraganian, R. P. (1993) *J. Biol. Chem.* **268,** 23318–23324
16. Hamawy, M. M., Mergenhagen, S. E., and Siraganian, R. P. (1993) *J. Biol. Chem.* **268,** 6851–6854
17. Kawakami, Y., Yao, L., Miura, T., Tsukada, S., Witte, O. N., and Kawakami, T. (1994) *Mol. Cell. Biol.* **14,** 5108–5113
18. Hamawy, M. M., Swaim, W. D., Minoguchi, K., de Feijter, A. W., Mergenhagen, S. E., and Siraganian, R. P. (1994) *J. Immunol.* **153,** 4655–4662
19. Minoguchi, K., Benhamou, M., Swaim, W. D., Kawakami, Y., Kawakami, T., and Siraganian, R. P. (1994) *J. Biol. Chem.* **269,** 16902–16908
20. Swaim, W. D., Minoguchi, K., Oliver, C., Hamawy, M. M., Kihara, H., Stephan, V., Berenstein, E. H., and Siraganian, R. P. (1994) *J. Biol. Chem.* **269,** 19466–19473
21. Jouvin, M. H. E., Adamczewski, M., Numerof, R., Letourneur, O., Vallé, A., and Kinet, J. P. (1994) *J. Biol. Chem.* **269,** 5918–5925
22. Pingel, J. T., and Thomas, M. L. (1989) *Cell* **58,** 1055–1065
23. Koretzky, G. A., Picus, J., Thomas, M. L., and Weiss, A. (1990) *Nature* **346,** 66–68
24. Kishihara, K., Penninger, J., Wallace, V. A., Kundig, T. M., Kawai, K., Wakeham, A., Timms, E., Pfeffer, K., Ohashi, P. S., Thomas, M. L., Furlonger, C., Paige, C. J., and Mak, T. W. (1993) *Cell* **74,** 143–156
25. Hook, W. A., Berenstein, E. B., Zinsser, F. U., Fischler, C., and Siraganian, R. P. (1991) *J. Immunol.* **147,** 2670–2676
26. Berger, S. A., Mak, T. W., and Paige, C. J. (1994) *J. Exp. Med.* **180,** 471–476
27. Zanke, B., Suzuki, H., Kishihara, K., Mizzen, L., Minden, M., Pawson, T., and Mak, T. W. (1992) *Eur. J. Immunol.* **22,** 235–239
28. Adachi, M., Sekiya, M., Isobe, M., Kumura, Y., Ogita, Z. I., Hinoda, Y., Imai, K., and Yachi, A. (1992) *Biochem. Biophys. Res. Commun.* **186,** 1607–1615
29. Streuli, M., Krueger, N. X., Tsai, A. Y., and Saito, H. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86,** 8698–8702
30. Nishikata, H., Oliver, C., Mergenhagen, S. E., and Siraganian, R. P. (1992) *J. Immunol.* **149,** 862–870
31. Barsumian, E. L., Isersky, C., Petrino, M. G., and Siraganian, R. P. (1981) *Eur. J. Immunol.* **11,** 317–323
32. Siraganian, R. P., and Hook, W. A. (1986) in *Manual of Clinical Laboratory Immunology* (Rose, N. R., Friedman, H., and Fahey, J. L., eds) 3rd Ed., pp. 675–684, American Society of Microbiology, Washington, D. C.
33. Benhamou, M., and Siraganian, R. P. (1992) *Immunol. Today* **13,** 195–197
34. Hamawy, M. M., Mergenhagen, S. E., and Siraganian, R. P. (1995) *Cell. Signal.*, in press
35. Keegan, A. D., and Paul, W. E. (1992) *Immunol. Today* **13,** 63–68
36. Frangioni, J. V., Beahm, P. H., Shifrin, V., Jost, C. A., and Neel, B. G. (1992) *Cell* **68,** 545–560
37. McLaughlin, S., and Dixon, J. E. (1993) *J. Biol. Chem.* **268,** 6839–6842
38. Gu, M., York, J. D., Warshawsky, I., and Majerus, P. W. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 5867–5871
39. Yang, Q., and Tonks, N. K. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 5949–5953
40. Moller, N. P. H., Moller, K. B., Lammers, R., Kharitonenkov, A., Sures, I., and Ullrich, A. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 7477–7481
41. Mauro, L. J., and Dixon, J. E. (1994) *Trends Biochem. Sci.* **19,** 151–155
42. Berridge, M. J. (1993) *Nature* **361,** 315–325
43. Benhamou, M., Stephan, V., Robbins, K. C., and Siraganian, R. P. (1992) *J. Biol. Chem.* **267,** 7310–7314
44. Feng, G. S., Hui, C. C., and Pawson, T. (1993) *Science* **259,** 1607–1611
45. Vogel, W., Lammers, R., Huang, J., and Ullrich, A. (1993) *Science* **259,** 1611–1614
46. Minoguchi, K., Kihara, H., Nishikata, H., Hamawy, M. M., and Siraganian, R. P. (1994) *Mol. Immunol.* **31,** 519–529
47. Yamashita, T., Mao, S. Y., and Metzger, H. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 11251–11255